bio-protocol Preprint

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Generation of E. coli strains

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An abbreviated version of this protocol was published in Science Advances in Mar 2021

Exonuclease VII repairs quinolone-induced damage by resolving DNA gyrase cleavage complexes

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Detailed protocol

Constructing NM1100

- Grow 5 ml MG1655 in LB to an OD600 ~ 0.4-0.6.
- Pellet the cells
- Resuspend the cells in 5 ml ice cold water
- Pellet in a refrigerated centrifuge
- Resuspend in 1 ml of ice cold water
- Pellet
- Repeat washes for a total of 4 times always on ice and cold water and refrigerated centrifuge
- Resuspend pellet in 50 ml ice cold water
- Transfer to cold 0.2 cm gap electroporation cuvette
- Add 100 ng of mini-I DNA Tet (https://redrecombineering.ncifcrf.gov/references-2/general-recombineering/court-2003-gene.pdf)
- Electroporate DNA on bacterial settings for electroporator
- Add 950 ml SOC media for recovery
- Incubate 1hr at 32 C
- Plate 10 ml and 200 ml on LB-Tet_{25}
- Incubate overnight at 32 C

Constructing NM50000

- Transform NM1100 with pBeloBac-$gyrA_{his}$ and plate on LB-Cm_{10} at 32 C overnight
- Take one colony and grow in LB-Cm_{10} to an OD600 ~ 0.4-0.6
- Induce the mini-I by shifting the culture to a 42 C water bath for 15 minutes
- Take the flask from 42 C directly into an ice water slurry and swirl continuously for 2 min
- Continue swirling on and off for up to 10 min.
- Pellet the cells in a refrigerated centrifuge at 4 C and wash as mentioned above (https://redrecombineering.ncifcrf.gov/references-2/general-recombineering/cpmb-2014.pdf)
- Cells are electrocompetent
- Electroporate with 100 ng of gyrA::zeo DNA.

gyrA::zeo
Two 60-mers were designed with one forward having 40 nt of homology upstream to gyrA and 20 to amplify a zeo antibiotic marker; while the reverse has 40 nt homology downstream and 20 nt to amplify the zeo marker. PCR product is cleaned and verified on gel to make sure it is a single band ~ 600 bp.

For additional questions, please feel free to contact me directly: majdalan@mail.nih.gov

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Huang, S. , Majdalani, N. and Pommier, Y. (2022). Generation of E. coli strains. Bio-protocol Preprint. bio-protocol.org/prep1597.
2. Huang, S. N., Michaels, S. A., Mitchell, B. B., Majdalani, N., Broeck, A. V., Canela, A., Tse-Dinh, Y., Lamour, V. and Pommier, Y.(2021). Exonuclease VII repairs quinolone-induced damage by resolving DNA gyrase cleavage complexes. Science Advances 7(10). DOI: 10.1126/sciadv.abe0384

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